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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Compositions for diagnosis and therapy of diseases associated with aberrant expression of futrins

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Compositions for diagnosis and therapy of diseases associated with aberrant expression of *Futrins*

The present invention relates to compositions useful for the diagnosis and therapy of diseases associated with aberrant expression of the genes encoding the proteins futrin1, 2, 3 and/or 4. These diseases include tumors of e.g. the breast, ovary, liver, uterus, cervix, colon, lung, ovary, rectum, testis, pancreas, bones and skin, as well as diseases involving muscle, bone, lipid and glucose metabolism, and obesity. The present invention also relates to a pharmaceutical composition containing a compound which is capable of modifying (a) the expression of the gene encoding Futrin1, 2, 3 and/or 4 or (b) the activity of Futrin1, 2, 3 and/or 4.

The Wnt signal cascade plays a crucial role as regards regulation of survival, proliferation and differentiation of cells during embryogenesis, and in the adult as shown, e.g., in *Drosophila, Xenopus* and mice (Nusse and Varmus, Cell 69 (1992), 1073-1087). Wnt-genes encode secretory glycoproteins which activate a well characterized signal cascade via a Wnt receptor called „frizzled„.

The Wnt signalling cascade and its components also play an important role in various diseases which makes it desirable to modulate its activity:

i) Cancer

Tumorigenesis represents a complex multistage process in which genetic changes and environmental factors are thought to deregulate the cellular processes that control cell proliferation and differentiation. Several studies indicate that an aberrant Wnt signal cascade is involved in the development of colon cancer, breast cancer and melanoma

(Pfeifer, Science, 275 (1997), 1752-1753; Polakis, Genes Dev. 14 (2000), 1837-1851). The first gene encoding a protein of the Wnt signal cascade, *int-1*, was isolated from mouse mammary tumor virus (MMTV) and it could be shown that it is an oncogene. It is thus well established that an aberrant regulation of the activity of Wnt and/or components of the Wnt signal cascade downstream of the Wnt signal, e.g., beta-catenin and APC, are involved in tumorigenesis.

ii) Bone disease

Wnt signals promote bone formation (e.g. Yang, Development, 130(2003), 1003-15; Fischer, J.Biol.Chem. 277 (2002) 30870-30878). Consistent with this notion, a gain-of-function mutation of the Wnt receptor LRP5 causes high bone disease (Boyden, et al., 346 (2002) N Engl J Med, 1513-21.; Little, et al., 70 (2002) Am J Hum Genet, 11-9.). Conversely, inactivating mutations in LRP5 leads to osteoporosis-pseudoglioma syndrome in humans (Kato, et al., 157 (2002) J Cell Biol, 303-14.; Gong, et al., 107 (2001) Cell, 513-23.).

iii) Eye disease

Inactivating mutation in the Wnt receptor LRP5 lead to pseudoglioma in humans and eye malformations in mice (Kato, et al., 157 (2002) J Cell Biol 303-314; Gong, et al., 107 (2001) Cell, 513-523).

iv) Kidney

Aberrant Wnt signalling is involved in renal fibrosis (Surendran, Am J Physiol Renal Physiol 282 (2002) 431-441) and polycystic kidney disease (Saadi-Kheddouci, Oncogene 20 (2001) 5972-5981).

v) Lipid and glucose metabolism, obesity

Deficiency of the Wnt receptor LRP5 in mice leads to increased plasma cholesterol levels in mice fed a high-fat diet, because of the decreased hepatic clearance of chylomicron remnants. In

addition, when fed a normal diet, LRP5-deficient mice show a markedly impaired glucose tolerance (Fujino, et al., 100 (2003) Proc Natl Acad Sci U S A, 229-234.) Administration of the LRP5 antagonist Dkk1 to mice reduces glucose uptake in various cell line and decreases fat deposition (WO 02/066509).

It is thus clear from the above that the Wnt signalling pathway is involved in a variety of human diseases. Yet, means for the therapy or diagnosis of diseases associated with a dis-regulated Wnt signal cascade are insufficiently available. Thus, the use of reliable diagnostic molecular markers would be helpful for an understanding of the molecular basis of diseases associated with an aberrant Wnt signal cascade. It can be expected that such markers are also useful for therapy and for the development of novel therapeutic avenues for treatment of Wnt signal cascade dependent diseases, as detailed above.

Thus, the technical problem underlying the present invention is to provide means for diagnosis and therapy of diseases associated with an aberrant Wnt signal cascade.

The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

During the experiments resulting in the present invention four genes, *futrin1, 2, 3* and *4*, could be identified the products of which are modulators of the Wnt pathway. Futrin2 was previously identified as hPWTSR (Chen et al., 29 (2002), Mol. Biol. Rep. 287-292), a protein of before unknown role or function, expressed in numerous cell types. Further, human Futrin1,2,3, and 4 were described as Stem Cell Growth Factor-Like Polypeptides, which are able to promote proliferation of hematopoietic stem cells (WO-A-01/77169 ; WO-A-01/07611).

In the present invention the following is shown for the first

time: 1) Futrins enhance Wnt signalling and this is of physiological relevance since inhibition of Futrin1 or 2 results in inhibition of the Wnt signal cascade. These data show that Futrins can be regarded as Wnt modulators. Thus, Futrins are useful for the diagnosis and the development of therapies for Wnt-LRP mediated diseases, including but not limited to tumor suppression, bone formation, cholesterol and glucose metabolism (including diabetes), obesity, kidney disease and eye disease. 2.) The data obtained show that Futrin2 is required for muscle formation. Thus, Futrin2 is useful for the diagnosis and the development of therapies for muscle related diseases, including muscle regeneration. 3.) The data show that Futrins are aberrantly expressed in a variety of human tumors. Thus, Futrins are useful for tumor diagnosis and the development of cancer therapies. For example it has been found out that in most of the tumors the expression of Futrins 1-3 is dramatically decreased (colon, stomach, lung, rectum tumors for Futrin1, breast, ovary, bladder, uterus, cervix, rectum tumors for Futrin2, uterus and cervix tumors for Futrin3). In a few cases the expression of Futrin 1-3 is upregulated (one case of stomach tumor for Futrin1 and 2, ovary tumor for Futrin3). Futrin4 shows very low level of expression in most of the tissues studied, except ovary.

Thus, the inhibition of the Wnt signal cascade by inhibiting the expression/activity of *Futrins* or by stimulating the expression/activity of the *Futrins* will have a therapeutic effect. Likewise, the activation of the Wnt signal cascade by decreasing the expression of *Futrin* and/or by repressing the activity of the polypeptide itself will have a therapeutic effect.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Identification of Xenopus Futrin1

TOP-FLASH reporter, vector DNA from pools of 250 clones each and pCSfrizzled8 were co-transfected. Luciferase reporter assays in 293T cells were carried out in 96 well plates in duplicates as described (Wu et al., Curr Biol 10 (2000), 1611-1614). Luciferase activity was normalized against cotransfected Renilla-luc activity using a commercial kit (Clonetech). RLU: relative luciferase units

Figure 2: Multisequence nucleic acid alignment of cDNAs encoding human Futrin1,2 3 and 4 and Xenopus Futrin1

Identical nucleotides are highlighted in black. All nucleic acid sequences begin with the translation initiator ATG codon indicated with an asterisk.

Figure 3: Multisequence amino acid alignment of human Futrin1,2 3 and 4 deduced from human cDNAs (see Figure 2)

Identical amino acids are highlighted in black, similar amino acids are in grey.

Figure 4: Futrins promote Wnt signalling

Cotransfection experiments in 293T cells. Wnt-responsive luciferase reporter assays were performed in 96 well plates in triplicates as described (Wu et al., Curr Biol 10 (2000), 1611-1614). Luciferase activity was normalized against Renilla activity using a commercial kit (Clonetech). Wnt1 = mouse Wnt1, fz8 = mouse frizzled8, Futrin1 = *xenopus* Futrin1, Wnt3A = mouse Wnt3A, (0.1ng) in A indicates amount of plasmid DNA transfected per well, RLU: relative luciferase units

Figure 5: Human Futrin1 and 2 are required for Wnt signalling

Hela cells were transfected in 24-well plates with the Wnt reporter 7LEF-Rev-fosLuc, pRL-TK and pSuper plasmids that produce either siRNA against human Futrin1 and 2 or a nonsense control. Three days after transfection, mouse Wnt3A conditioned medium was added to the culture to stimulate Wnt

signalling. 24 hours later, luciferase activity was determined.

Figure 6: Futrin2 is required for muscle formation

A. Depletion of Futrin1 protein causes downregulation of early muscle markers and muscle defects. (A,C,E,G,I,K) Embryos injected with control morpholino oligo (5ng), with Fut1-Mo (5ng). Oligos were mixed with lineage tracer mRNA (LacZ) (blue staining) in (C,D,E,F,G,H) or preprolactin (ppl) RNA, visualised by *in situ* hybridisation (red staining) (A,B,J,K,L). In situ hybridization with probes to Xmyf5 (A,B), XmyoD (C,D), Xbra (E,F), Xnot (G,H) and muscle actin (I-L) (magenta staining). Embryos in K and L are cut transversally. Note reduction of muscle in L (right side).

B. Fut1-Mo act specifically to inhibit translation of their cognate DNA constructs when overexpressed in embryos. mRNA (C-terminal myc tagged fut1) was injected equatorially in both blastomeres at 2-cell stage embryos. The same embryos were then injected at the 8-cell stage with 5ng of Fut1-Mo (lane3) or control morpholino (lane2) in all vegetal blastomeres and harvested at stage 11. Tagged Futrin1 protein was then visualised with a-myc anibody.

C. Rescue of muscle marker reduction caused by Fut1-Mo by coinjection of XFut1 mRNA containing point mutations in the 5'region corresponding to morpholino oligo. Embryos were coinjected with 5 ng of Fut1-Mo (1,2) with 50 pg of ppl or XFut1 mRNA radially in one blastomere at 4-cell stage.
Expression of MyoD (1,3) and Myf5 (2,4) were analysed by in situ hybridization. All embryos were grouped into 3 classes (examples for MyoD are shown on the bottom of the figure): embryos with expression level on the injected side from 1-30%(class A); 30-70%(class B), and 70-

100%(class C) from normal level. Bars represent the percentage of the embryos corresponding to type A,B or C. (n for Fut-Mo + ppl: 57 embryos for MyoD, 45 embryos for Myf5; n for Fut-Mo + mRNA Ffut1: 39 embryos for MyoD and 41 embryo for Myf5).

Figure 7: Futrin expression is deregulated in various human tumors

The expression of *Futrin1, 2, 3 and-4* or ubiquitin (to show equal loading) was analysed by radioactive hybridisation on arrayed mRNAs (Clontech, Cancer Profiling Array II) from normal and cancerous tissue samples from different patients. Abbreviations N, normal tissues; T, tumor tissues.

The present invention relates to a diagnostic composition comprising:

(a) at least one nucleic acid molecule comprising the nucleotide sequence encoding Futrin1, 2, 3 or 4 as depicted in Figure 2; and/or

(b) at least one polypeptide molecule comprising the amino acid sequence encoding Futrin1,2,3 or 4 as depicted in Figure 3; and/or

(c) at least one nucleic acid molecule the complementary strand of which hybridizes to a nucleic acid molecule of (a) and which encodes a polypeptide with the biological activity of futrin1, 2, 3 or 4; and/or

(d) at least one fragment of (a), (b) or (c) having the biological activity of futrin1, 2, 3 or 4;

(e) at least one nucleic acid molecule the sequence of which differs from the sequence of the nucleic acid molecule of (a), (c) or (d) due to the degeneracy of the genetic code, and/or

(f) at least one ligand capable of specifically binding to the molecule of (a), (b), (c), (d) or (e).

As used herein the term „polypeptide„ not only refers to polypeptids encoded by the nucleotide/amino acid sequences as depicted in Figure 2 and/or 3 but also to polypeptides differing in amino acid sequence due to insertion, deletion and/or substitution of one ore more amino acids and showing at least one biological activity of a Futrin, e.g. the ability to promote Wnt signalling. Preferably, the related nucleic acids and/or polypeptides are nucleic acids and/or polypeptides the sequence of which shows an identity of at least 40%, in particular an identity of at least 65%, preferably of at least 80% and, particularly preferred, of at least 90% to the amino acid sequences of the polypeptides encoded by the nucleotide sequences shown in Figure 2.

The nucleic acid molecules useful as probes can be both DNA and RNA molecules, preferably they are single-stranded DNA molecules. They can be isolated from natural sources or can be synthesized according to know methods.

As a hybridization probe nucleic acid molecules can be used, for example, that have a nucleotide sequence which is exactly or basically complementary to a nucleotide sequence as depicted in Figure 2 and 3, respectively, or parts of these sequences. The fragments used as hybridization probe can be synthetic fragments that were produced by means of conventional synthetic methods.

As used herein, the term „hybridizing„ relates to hybridization under conventional hybridization conditions, preferably under stringent conditions as described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. However, in certain cases, a hybridizing nucleic acid molecule can also be detected at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily

accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency), salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°Cin a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 9.2M NaH_2PO_4; 0.02M EDTA, pH7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA, following by washes at 50°C with 1 X SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC). Variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

The term „ligand„ as used herein refers to any molecule which is capable of specifically binding to Futrin1,2,3, or 4, thus allowing to determine the level of receptor molecules. Examples of such molecules include antibodies, oligonucleotides, proteins or small molecules. The molecule can be the natural ligand of Futrins, or can be closely related to said ligand, e.g., a fragment of the ligand, or a natural substrate, a structural or functional mimetic; see, e.g., Coligan, Current Protocols in Immunology 1(2) (1991); Chapter 5. In either case, the molecule can be isolated or rationally designed using known techniques; see also infra.

Preferably, the ligand is an antibody. The term „antibody„, preferably, relates to antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specifities, as well as distinct monoclonal antibody preparations. Monoclonal antibodies are made from an antigen containing Futrin1,2,3, or 4 or fragments thereof by methods

well known to those skilled in the art (see, e.g., Köhler et al., Nature 256 (1975), 495). As used herein, the term „antibody„ (Ab) or „monoclonal antibody„ (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab') 2 fragments) which are capable of specifically binding to Futrin1, 2, 3 and/or 4. Fab and f(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J. Nucl. Med. 24: 316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimerical, single chain, and humanized antibodies.

For certain purposes, e.g. diagnostic methods, the nucleic acid molecule used as probe or the ligand, e.g., antibody, can be detectably labeled, for example, with a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

The nucleic acid molecules can be used, for example, as probes or primers in the diagnostic assays described below and allow, e.g., the analysis of the expression of Futrin1,2,3, or -4 by determining the mRNA level or the determination of mutations within the coding region or regulatory regions leading to polypeptide molecules with altered, e.g. destroyed, activity, or leading to altered expression. Preferably, the nucleic acid molecules are oligonucleotides having a length of at least 10, in particular of at least 15 and particularly preferred of at least 50 nucleotides. These nucleic acid molecules of the invention can also be used, for example, as primers for a PCR reaction.

The present invention also relates to the use of a nucleic acid molecule or ligand as defined above for the preparation of a

diagnostic composition for the diagnosis of a disease associated with (a) aberrant expression of Futrin1,2,3, or -4 and/or (b) aberrant activity of a Futrin1,2,3, or -4 polypeptide.

In a preferred embodiment, the target to which the nucleic acid molecule hybridizes is an mRNA.

The present invention also provides a method of diagnosing a disease associated with (a) aberrant expression of Futrin1,2,3, or -4 and/or (b) aberrant activities or amounts of a Futrin1,2,3, or -4 polypeptide in a subject comprising:

(a) determining (a) the amount of expression of Futrin1,2,3, or -4 and/or (b) the amount of biologically active Futrin1, 2, 3 and/or 4 polypeptide in a biological sample; and

(b) diagnosing a disease associated with (a) aberrant expression of *Futrin1, 2, 3 and/or 4* and/or (b) aberrant activites or amounts of a Futrin1, 2, 3 and/or 4 polypeptide or a risk for the development of such diasease based on an altered amount of expression of *Futrin1, 2, 3 and/or 4* and/or (b) altered activities or amounts of biologically active Futrin1, 2, 3 and/or 4 polypeptide compared to a control.

Suitable assay formats are well known to the person skilled in the art and, in addition, described below. Suitable positive control samples expressing human Futrin proteins are, e.g., HEK 293 cells.

The Futrin1,2,3, or 4 polypeptide or the corresponding mRNA, e.g. in biological fluids or tissues, may be detected directly in situ, e.g. by in situ hybridization or it may be isolated from other cell components by common methods known to those skilled in the art before contacting with a probe. Detection methods include Northern Blot analysis, RNase protection, in situ methods, e.g. in situ hybridization, in vitro amplification methods (PCR, LCR, QRNA replicase or RNA-

transcription/amplification (TAS, 3SR), reverse dot blot disclosed in EP-B1 O 237 362), immunoassays, Western Blot and other detection assays that are known to those skilled in the art.

The probe (e.g. a specific antibody or specific oligonucleotide) of the diagnostic composition can be detectably labeled. In a preferred embodiment, said diagnostic composition contains an anti- Futrin1, 2, 3 and/or 4 antibody and allows said diagnosis, e.g., by ELISA and contains the antibody bound to a solid support, for example, a polystyrene microtiter dish or nitrocellulose paper, using techniques known in the art. Alternatively, said diagnostic compositions are based on a RIA and contain said antibody marked with a radioactive isotope. Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium rhodamine, and biotin. In addition to assaying Futrin levels in a biological sample, the polypeptide can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system

used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific Futrin polypeptide. In vivo tumor imaging is, e.g., described in S.W. Burchiel et al., „Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments„. (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B.A. Rhodes, eds., Masson Publishing Inc. (1982)).

In a further aspect, the present invention, relates to a method for identifying a binding partner to a Futrin1, 2, 3 and/or -4 polypeptide comprising:

(a) contacting said polypeptide with a compound to be screened; and

(b) determining whether the compound effects an activity of the polypeptide.

The invention also includes a method of identifying compounds which bind to a Futrin1, 2, 3 and/or 4 polypeptide comprising the steps of:

(a) incubating a candidate binding compound with said polypeptide; and

(b) determining if binding has occurred.

Futrin1, 2, 3 or -4 polypeptides may be used to screen for proteins or other compounds that bind to Futrin1, 2, 3 or -4 or for proteins or other compounds to which Futrin1, 2, 3 and/or 4 bind. The binding of Futrin1, 2, 3 or -4 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of Futrin1, 2, 3 or 4 or the molecule bound. Examples of such molecules include

antibodies, oligonucleotides, proteins (e.g., ligands), or small molecules.

Preferably, the molecule is closely related to the natural ligand of Futrin1, 2, 3 or -4, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic; see, e.g., Coligan, Current Protocols in Immunology 1(2) (1991); Chapter 5.

Preferably, the screening for these molecules involves producing appropriate cells which express Futrin1, 2, 3 and/or 4 either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing Futrin1, 2, 3 and/or -4 (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of Futrin1, 2, 3 and/or -4.

The assay may simply test binding of a candidate compound to Futrin1, 2, 3 and/or 4, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to Futrin1, 2, 3 and/or 4. Suitable assays to analyze the activity of Futrin1, 2, 3 and/or 4 include Wnt-inducible luciferase reporter assays in transfected HEK 293 cells, where Futrin1, 2, 3 and/or 4 synergizes with Wnt to enhance a Wnt-induced signal, such as is shown in Figure 4.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing Futrin1, 2, 3 and/or 4, measuring

Futrin/molecule activity or binding, and comparing the Futrin/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure Futrin1, 2, 3 and/or 4 level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure Futrin1, 2, 3 and/or 4 level or activity by either binding, directly or indirectly, to Futrin1, 2, 3 and/or 4 or by competing with Futrin1, 2, 3 and/or 4 for a substrate. All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., elimination of a tumor, support of regenerative processes etc.) by modulating, preferably activating the Futrin1, 2, 3 and/or 4 molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of Futrin1, 2, 3 and/or 4 from suitably manipulated cells or tissues.

Moreover, the invention includes a method of identifying activators/agonists or inhibitors/antagonists of a Futrin1, 2, 3 and/or 4 polypeptide comprising the steps of:

(a) incubating a candidate compound with said polypeptide;
(b) assaying a biological activity, and
(c) determining if a biological activity of said polypeptide has been altered.

Suitable assays to analyze the activity of Futrin1, 2, 3 and/or 4 include Wnt-inducible luciferase reporter assays in transfected HEK 293 cells, where Futrin1, 2, 3 and/or 4 synergizes with Wnt to enhance a Wnt-induced signal, such as is shown in Figure 4.

In a further embodiment, the present invention relates to method of identifying and obtaining a drug candidate for therapy of diseaseas associated with (a) aberrant expression of

Futrin1, 2, 3 and/or 4 and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 polypeptide comprising the steps of

(a) contacting a Futrin1, 2, 3 and/or 4 polypeptide or a cell expressing said polypeptide, and optionally the corresponding ligand(s), in the presence of components capable of providing a detectable signal in response to binding to said drug candidate to be screened; and

(b) detecting presence or absence of a signal or increase of the signal generated, wherein the presence or increase of the signal is indicative for a putative drug.

Suitable assays to analyze the activity of Futrin1, 2, 3 and/or 4 include Wnt-inducible luciferase reporter assays in transfected HEK 293 cells, where Futrin1, 2, 3 and/or 4 synergizes with Wnt to enhance a Wnt-induced signal, such as is shown in Figure 4.

The drug candidate may be a single compound or a plurality of compounds. The term "plurality of compounds" in a method of the invention is to be understood as a plurality of substances which may or may not be identical.

Said compound or plurality of compounds may be chemically synthesized or microbiologically produced and/or comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compound(s) may be known in the art but hitherto not known to be capable of suppressing or activating Futrin1, 2, 3 and/or 4 polypeptides. The reaction mixture may be a cell free extract or may comprise a cell or tissue culture. Suitable set ups for the method of the invention are known to the person skilled in the art and are, for example, generally described in Alberts et al., Molecular Biology of the Cell, third edition (1994) and in the appended examples. The plurality of compounds may be, e.g., added to the reaction mixture, culture medium, injected into a cell or otherwise applied to a transgenic animal. The

cell or tissue that may be employed in the method of the invention preferably is a host cell, mammalian cell or non-human transgenic animal.

If a sample containing a compound or a plurality of compounds is identified in the method of the invention, then it is either possible to isolate the compound from the original sample identified as containing the compound capable of suppressing or activating a Futrin1, 2, 3 and/or 4 polypeptide, or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical.

Several methods are known to the person skilled in the art for producing and screening large libraries to identify compounds having specific affinity for a target. These methods include the phage-display method in which randomized peptides are displayed from phage and screened by affinity chromatography to an immobilized receptor; see, e.g., WO 91/17271, WO 92/01047, US-A-5,223,409. In another approach, combinatorial libraries of polymers immobilized on a chip are synthesized using photolithography; see, e.g., US-A-5,143,854, WO 90/15070 and WO 92/10092. The immobilized polymers are contacted with a labeled receptor and scanned for label to identify polymers binding to the receptor. The synthesis and screening of peptide libraries on continuous cellulose membrane supports that can be used for identifying binding ligands of the Futrin1, 2, 3 and/or -4 polypeptides and, thus, possible

inhibitors and activators is described, for example, in Kramer, Methods Mol. Biol. 87 (1998), 25-39. This method can also be used, for example, for determining the binding sites and the recognition motifs in the Futrin1, 2, 3 and/or -4 polypeptide. In like manner, the substrate specificity of the DnaK chaperon was determined and the contact sites between human interleukin-6 and its receptor; see Rudiger, EMBO J. 16 (1997), 1501-1507 and Weiergraber, FEBS Lett. 379 (1996), 122-126, respectively. Furthermore, the above-mentioned methods can be used for the construction of binding supertopes derived from the Futrin1, 2, 3 or 4 polypeptide. A similar approach was successfully described for peptide antigens of the anti-p24 (HIV-1) monoclonal antibody; see Kramer, Cell 91 (1997), 799-809. A general route to fingerprint analyses of peptide-antibody interactions using the clustered amino acid peptide library was described in Kramer, Mol. Immunol. 32 (1995), 459-465. In addition, antagonists of a Futrin1, 2, 3 and/or 4 polypeptide can be derived and identified from monoclonal antibodies that specifically react with a Futrin1, 2, 3 and/or 4 polypeptide in accordance with the methods as described in Doring, Mol. Immunol. 31 (1994), 1059-1067.

All these methods can be used in accordance with the present invention to identify activators/agonists and inhibitors/antagonists of a Futrin1, 2, 3 and/or 4 polypeptide.

Various sources for the basic structure of such an activator or inhibitor can be employed and comprise, for example, mimetic analogs of a Futrin1, 2, 3 and/or 4 polypeptide. Mimetic analogs of a Futrin1, 2, 3 and/or 4 polypeptide or biologically active fragments thereof can be generated by, for example, substituting the amino acids that are expected to be essential for the biological activity with, e.g., stereoisomers, i.e. D-amino acids; see e.g., Tsukida, J. Med. Chem. 40 (1997), 3534-3541. Furthermore, in case fragments are used for the design of

biologically active analogs pro-mimetic components can be incorporated into a peptide to reestablish at least some of the conformational properties that may have been lost upon removal of part of the original polypeptide; see, e.g., Nachman, Regul. Pept. 57 (1995), 359-370. Furthermore, a Futrin1, 2, 3 and/or 4 polypeptide can be used to identify synthetic chemical peptide mimetics that bind to or can function as a ligand, substrate or binding partner of said polypeptide(s) as effectively as does the natural polypeptide; see, e.g., Engleman, J. Clin. Invest. 99 (1997), 2284-2292. For example, folding simulations and computer redesign of structural motifs of a Futrin1, 2, 3 and/or 4 polypeptide can be performed using appropriate computer programs (Olszewski, Proteins 25 (1996), 286-299; Hoffman, Comput. Appl. Biosci. 11 (1995), 675-679). Computer modeling of protein folding can be used for the conformational and energetic analysis of detailed peptide and protein models (Monge, J. Mol. Biol. 247 (1995), 995-1012; Renouf, Adv. Exp. Med. Biol. 376 (1995), 37-45). In particular, the appropriate programs can be used for the identification of interactive sites of a Futrin1, 2, 3 and/or 4 polypeptide and its ligand or other interacting proteins by computer assistant searches for complementary peptide sequences (Fassina, Immunomethods 5 (1994), 114-120. Further appropriate computer systems for the design of protein and peptides are described in the prior art, for example in Berry, Biochem. Soc. Trans. 22 (1994), 1033-1036; Wodak, Ann. N. Y. Acad. Sci. 501 (1987), 1-13; Pabo, Biochemistry 25 (1986), 5987-5991. The results obtained from the above-described computer analysis can be used for, e.g., the preparation of peptide mimetics of a Futrin1, 2, 3 and/or 4 polypeptide or fragments thereof. Such pseudopeptide analogues of the natural amino acid sequence of the protein may very efficiently mimic the parent protein (Benkirane, J. Biol. Chem. 271 (1996), 33218-33224). For example, incorporation of easily available achiral ω -amino acid residues into a Futrin1, 2, 3 or -4 polypeptide or a fragment thereof results in the substitution of amide bonds by polymethylene units of an aliphatic chain, thereby providing a

convenient strategy for constructing a peptide mimetic (Banerjee, Biopolymers 39 (1996), 769-777). Superactive peptidomimetic analogues of small peptide hormones in other systems are described in the prior art (Zhang, Biochem. Biophys. Res. Commun. 224 (1996), 327-331). Appropriate peptide mimetics of a Futrin1, 2, 3 and/or 4 polypeptide can also be identified by the synthesis of peptide mimetic combinatorial libraries through successive amide alkylation and testing the resulting compounds, e.g., for their binding and immunological properties. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, Methods in Enzymology 267 (1996), 220-234 and Dorner, Bioorg. Med. Chem. 4 (1996), 709-715. Furthermore, a three-dimensional and/or crystallographic structure of a Futrin1, 2, 3 and/or 4 polypeptide can be used for the design of peptide mimetic inhibitors of the biological activity of the polypeptide (Rose, Biochemistry 35 (1996), 12933-12944; Rutenber, Bioorg. Med. Chem. 4 (1996), 1545-1558).

It is also well known to the person skilled in the art, that it is possible to design, synthesize and evaluate mimetics of small organic compounds that, for example, can act as a substrate or ligand to a Futrin1, 2, 3 and/or 4 polypeptide. For example, it has been described that D-glucose mimetics of hapalosin exhibited similar efficiency as hapalosin in antagonizing multidrug resistance assistance-associated protein in cytotoxicity; see Dinh, J. Med. Chem. 41 (1998), 981-987.

The nucleic acid molecule encoding a Futrin1, 2, 3 and/or 4 polypeptide can also serve as a target for activators and inhibitors. Activators may comprise, for example, proteins that bind to the mRNA of a gene encoding a Futrin1, 2, 3 and/or 4 polypeptide, thereby stabilizing the native conformation of the mRNA and facilitating transcription and/or translation, e.g., in like manner as Tat protein acts on HIV-

RNA. Furthermore, methods are described in the literature for identifying nucleic acid molecules such as an RNA fragment that mimics the structure of a defined or undefined target RNA molecule to which a compound binds inside of a cell resulting in retardation of cell growth or cell death; see, e.g., WO 98/18947 and references cited therein. These nucleic acid molecules can be used for identifying unknown compounds of pharmaceutical interest, and for identifying unknown RNA targets for use in treating a disease. These methods and compositions can be used in screening for novel or for identifying compounds useful to alter expression levels of polypeptids encoded by a nucleic acid molecule. Alternatively, for example, the conformational structure of the RNA fragment which mimics the binding site can be employed in rational drug design to modify known drugs to make them bind more avidly to the target. One such methodology is nuclear magnetic resonance (NMR), which is useful to identify drug and RNA conformational structures. Still other methods are, for example, the drug design methods as described in WO 95/35367, US-A-5,322,933, where the crystal structure of the RNA fragment can be deduced and computer programs are utilized to design novel binding compounds.

The compounds which can be tested and identified according to a method of the invention may be expression libraries, e.g., cDNA expression libraries, peptides, proteins, nucleic acids, antibodies, small organic compounds, hormones, peptidomimetics, PNAs or the like (Milner, Nature Medicine 1 (1995), 879-880; Hupp, Cell 83 (1995), 237-245; Gibbs, Cell 79 (1994), 193-198 and references cited supra). Furthermore, genes encoding a putative regulator of a Futrin1, 2, 3 and/or 4 polypeptide and/or which excert their effects up- or downstream a Futrin1, 2, 3 and/or 4 polypeptide may be identified using, for example, insertion mutagenesis using, for example, gene targeting vectors known in the art. Said compounds can also be functional derivatives or analogues of

known inhibitors or activators. Such useful compounds can be for example transacting factors which bind to a Futrin1, 2, 3 and/or 4 polypeptide or regulatory sequences of the gene encoding it. Identification of transacting factors can be carried out using standard methods in the art (see, e.g., Sambrook, supra). To determine whether a protein binds to the protein itself or regulatory sequences, standard native gel-shift analyses can be carried out. In order to identify a transacting factor which binds to the protein or regulatory sequence, the protein or regulatory sequence can be used as an affinity reagent in standard protein purification methods, or as a probe for screening an expression library. The identification of nucleic acid molecules which encode polypeptides which interact with a Futrin1, 2, 3 and/or 4 polypeptide described above can also be achieved, for example, as described in Scofield (Science 274 (1996), 2063-2065) by use of the so-called yeast "two-hybrid system". In this system the Futrin1, 2, 3 or 4 polypeptide or a smaller part thereof is linked to the DNA-binding domain of the GAL4 transcription factor. A yeast strain expressing this fusion polypeptide and comprising a lacZ reporter gene driven by an appropriate promoter, which is recognized by the GAL4 transcription factor, is transformed with a library of cDNAs which will express plant proteins or peptides thereof fused to an activation domain. Thus, if a peptide encoded by one of the cDNAs is able to interact with the fusion peptide comprising a peptide of a Futrin1, 2, 3 and/or 4 polypeptide, the complex is able to direct expression of the reporter gene. In this way the nucleic acid molecules encoding Futrin1, 2, 3 and 4, respectively, and the encoded peptide can be used to identify peptides and proteins interacting with a Futrin1, 2, 3 and/or 4 polypeptide.

Once the transacting factor is identified, modulation of its binding to or regulation of expression of a Futrin1, 2, 3 and/or 4 polypeptide can be pursued, beginning with, for example, screening for inhibitors against the binding of the

transacting factor to a Futrin1, 2, 3 or 4 polypeptide. Activation or repression of a Futrin1, 2, 3 and/or 4 polypeptide could then be achieved in animals by applying the transacting factor (or its inhibitor) or the gene encoding it, e.g. in an expression vector. In addition, if the active form of the transacting factor is a dimer, dominant-negative mutants of the transacting factor could be made in order to inhibit its activity. Furthermore, upon identification of the transacting factor, further components in the signal cascade leading to activation (e.g. signal transduction) or repression of a gene involved in the control of a Futrin1, 2, 3 and/or 4 polypeptide then can be identified. Modulation of the activities of these components can then be pursued, in order to develop additional drugs and methods for modulating the metabolism of protein degradation in animals. Thus, the present invention also relates to the use of the two-hybrid system as defined above for the identification of activators or inhibitors of a Futrin1, 2, 3 and/or 4 polypeptide.

The compounds isolated by the above methods also serve as lead compounds for the development of analog compounds. The analogs should have a stabilized electronic configuration and molecular conformation that allows key functional groups to be presented to a Futrin1, 2, 3 and/or 4 polypeptide or its ligand in substantially the same way as the lead compound. In particular, the analog compounds have spatial electronic properties which are comparable to the binding region, but can be smaller molecules than the lead compound, frequently having a molecular weight below about 2 kD and preferably below about 1 kD. Identification of analog compounds can be performed through use of techniques such as self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics analysis. Computer programs for implementing these techniques are available; e.g., Rein, Computer-Assisted Modeling of Receptor-Ligand Interactions (Alan Liss, New York, 1989). Methods for the preparation of chemical derivatives and

analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art; see also supra. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used, for example, according to the methods described above.

Once the described compound has been identified and obtained, it is preferably provided in a therapeutically acceptable form.

Accordingly, the present invention also relates to a pharmaceutical composition comprising a nucleic acid molecule encoding a Futrin1, 2, 3 and/or 4 polypeptide, a Futrin1, 2, 3 and/or 4 polypeptide itself, recombinant vector (for examples, see below), antibody, activator/agonist, inhibitor/antagonist and/or binding partner of a Futrin1, 2, 3 and/or 4 polypeptide and a pharmaceutically acceptable excipient, diluent or carrier.

Preferably, for therapeutic purposes, the Futrin1, 2, 3 and/or 4 polypeptide is recombinantly produced by use of the nucleic acid sequences shown in Figures 1 and 2. Suitable vectors for recombinant expression are known to the person skilled in the art. Preferably, they are plasmids, cosmids, viruses, bacteriophages and other vectors usually used in the field of genetic engineering. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in mammalian cells and baculovirus-derived vectors for expression in insect cells. Preferably, the nucleic acid molecule of the invention is operatively linked to the regulatory elements in the

recombinant vector of the invention that guarantee the transcription and synthesis of an mRNA in prokryotic and/or eukaryotic cells that can be translated. The nucleotide sequence to be transcribed can be operably linked to a promoter like a T7, metallothionein I or polyhedrin promoter. The host cells used for recombinant expression are prokaryotic or eukaryotic cells, for example mammalian cells, bacterial cells, insect cells or yeast cells. The polypeptide is isolated from the cultivated cells and/or the culture medium. Isolation and purification of the recombinantly produced polypeptide may be carried out by conventional means including preparative chromatography and affinity and immunological separations using, e.g., an anti-Futrin1, 2, 3 or -4 antibody, or, e.g., can be substantially purified by the one-step method described in Smith and Johnson, Gene 67; 31-40 (1988).

Examples of suitable pharmaceutical carriers etc. are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Such carriers can be formulated by conventional methods and can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g. by intravenous, intraperetoneal, subcutaneous, intramuscular, topical or intradermal administration. The route of administration, of course, depends on the nature of the disease and the kind of compound contained in the pharmaceutical composition. The dosage regimen will be determined by the attending physician and other clinical factors. As is well known in the medical arts, dosages for any one patient depends on many factors, including the patient's size, body surface area, age, sex, the particular compound to be administered, time and route of administration, the kind and stage of the disease, e.g., tumor, general health and other drugs being administered concurrently.

The delivery of the nucleic acid molecules encoding a Futrin1, 2, 3 and/or 4 polypeptide can be achieved by direct application or, preferably, by using a recombinant expression vector such as a chimeric virus containing these compounds or a colloidal dispersion system. Direct application to the target site can be performed, e.g., by ballistic delivery, as a colloidal dispersion system or by catheter to a site in artery. The colloidal dispersion systems which can be used for delivery of the above nucleic acid molecules include macromolecule complexes, nanocapsules, microspheres, beads and lipid-based systems including oil-in-water emulsions (mixed), micelles, liposomes and lipoplexes, The preferred colloidal system is a liposome. Organ-specific or cell-specific liposomes can be used in order to achieve delivery only to the desired tissue. The targeting of liposomes can be carried out by the person skilled in the art by applying commonly known methods. This targeting includes passive targeting (utilizing the natural tendency of the liposomes to distribute to cells of the RES in organs which contain sinusoidal capillaries) or active targeting (for example by coupling the liposome to a specific ligand, e.g., an antibody, a receptor, sugar, glycolipid, protein etc., by well known methods). In the present invention monoclonal antibodies are preferably used to target liposomes to specific tissues, e.g. tumor tissue, via specific cell-surface ligands.

Preferred recombinant vectors useful for gene therapy are viral vectors, e.g. adenovirus, herpes virus, vaccinia, or, more preferably, an RNA virus such as a Retrovirus. Even more preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of such retroviral vectors which can be used in the present invention are: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV) and Rous sarcoma virus (RSV). Most preferably, a non-human primate retroviral vector is employed, such as the gibbon ape leukemia virus (GaLV),

providing a broader host range compared to murine vectors. Since recombinant retroviruses are defective, assistance is required in order to produce infectious particles. Such assistance can be provided, e.g., by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. Suitable helper cell lines are well known to those skilled in the art. Said vectors can additionally contain a gene encoding a selectable marker so that the transduced cells can be identified. Moreover, the retroviral vectors can be modified in such a way that they become target specific. This can be achieved, e.g., by inserting a polynucleotide encoding a sugar, a glycolipid, or a protein, preferably an antibody. Those skilled in the art know additional methods for generating target specific vectors. Further suitable vectors and methods for in vitro- or in vivo-gene therapy are described in the literature and are known to the persons skilled in the art; see, e.g., WO 94/29469 or WO 97/00957.

In order to achieve expression only in the target organ, e.g., a tumor to be treated, the nucleic acid molecules encoding a Futrin1, 2, 3 and/or 4 polypeptide can be linked to a tissue specific promoter and used for gene therapy. Such promoters are well known to those skilled in the art (see e.g. Zimmermann et al., (1994) Neuron 12, 11-24; Vidal et al.; (1990) EMBO J. 9, 833-840; Mayford et al., (1995), Cell 81, 891-904; Pinkert et al., (1987) Genes & Dev. 1, 268-76).

The present invention also relates to the use of the above compounds of the invention for the preparation of a pharmaceutical composition for treatment of a disease associated with (a) aberrant expression of Futrin1, 2, 3 and/or 4 and/or genes involved into the Wnt signal cascade, and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 and/or a polypeptide involved into the Wnt signal

cascade. In a preferred embodiment, said disease is a kidney, bone or muscle disease or tumor, preferably breast cancer, a colon carcinoma or a melanoma.

Finally, the present invention relates to the use of a nucleotide molecule encoding a polypeptide having a biological activity of Futrin1, 2, 3 and/or 4, a Futrin1, 2, 3 and/or 4 polypeptide, an activator/agonist of a Futrin1, 2, 3 and/or 4 polypeptide or binding partner of said polypeptide(s) for the preparation of a pharmaceutical composition for inhibiting the Wnt signal cascade which might be useful for supporting regenerative processes in a patient, e.g. growth of tissue like muscle, bone, hair, etc.

The following examples illustrate the invention.

Example 1

Isolation of a cDNA encoding Xenopus Futrin1

A Xenopus adult eye cDNA library in the expression vector pCS2+ was used to prepare pools of about 250 colonies, and plasmid DNA from each pool was transiently transfected into 293T cells together with the Wnt receptor frizzled8, the Wnt reporter TOP-FLASH (Korinek et al. Science 275 (1997) 1784-1787) and Renilla-luciferase for normalization, in 96-well plates using FuGENE 6 (Roche, Basel). After 24 hours relative luciferase activity was determined. One pool yielding a signal above background was identified (Figure 1)and a gene harboring this activity was isolated from the pool by sib selection. Sequencing analysis showed it represents Xenopus futrin1. Database searches revealed four closely related genes in human, called hfutrin1, 2, 3 and 4 (Figures 2-3).

Example 2

Futrins promote Wnt signalling

Xenopus Futrin1 and human Futrin1, 2, and 3 are able to stimulate Wnt-responsive reporter expression in HEK 293T cells when provided by transient transfection (Figure 4A). In addition, they are able to enhance reporter expression induced by Wnt (mouse Wnt1/3A) synergistically (Figure 4B). Cotransfection experiments in HEK239T cells were carried out with the indicated genes and the Wnt reporter TOP-FLASH (Korinek et al. Science 275 (1997) 1784-1787) and Renilla-luciferase for normalization, in 96-well plates using FuGENE 6 (Roche). After 24 hours relative luciferase activity was determined.

Example 3

Futrins are required for full Wnt signalling

To test the requirement of Futrins in Wnt signalling, siRNA mediated gene knock-out was utilized (Brummelkamp et al., Science. 2002, 296(5567):550-3). Hela cells were transfected using Lipofectamine Plus with 80ng Wnt reporter 7LEF-Rev-fosLuc, 10 ng pRL-TK (Promega) and 300 ng pSuper constructs (Brummelkamp et al.) that produce either siRNA against human Futrin1 and 2, or a nonsense control. 7LEF-Rev-fosLuc reporter construct containing seven LEF binding sites in front of minimal fos promoter followed by firefly luciferase ORF was kindly provided by R. Grosschedl (Howard Hughes Medical Institute). pSuper constructs contain 19-nucleotide sequences from human Futrin1 (sequence: TCCCATTTGCAAGGGTTGT), human Futrin2 (sequence: AGCTGACTGTGATACCTGT) or control nonsense sequence (ACTACCGTTGTTATAGGTG).

One day after transfection medium was changed from 10% to 0.5% FCS. Three days after transfection, mouse Wnt3A conditioned

medium or control medium from 293 cells was added to the culture to stimulate Wnt signalling. 24 hours later, luciferase activity was determined. As shown in Figure 5, Hela cells show reduced levels of Futrin1 and 2, and Wnt signalling dropped by 50%, indicating that Futrins are required for full Wnt signalling. This effect can be efficiently rescued by 5 ng recombinant mouse Futrin1, attesting its specificity. Data are normalized to Renilla luciferase activity.

Example 4

Futrin1 regulates muscle formation

Loss-of-function analysis in Xenopus embryos show that Futrin1 is required for muscle formation. Injections of antisence morpholino oligo against futrin1 (Fut1-Mo) cause downregulation of early muscle markers MyoD anf Myf5 and induce muscle defects (Figure 6A). The specificity of this effect was documented, first, by the ability of Fut1-Mo to inhibit translation of the cognate DNA construct when overexpressed in embryos (Figure 6B) and, second, by the ability of XFut1 mRNA to rescue the effect of Fut1-Mo (Figure 6C).

Example 5

Futrin expression is deregulated in human tumors

The expression of Futrin1-4 in various normal and cancerous human tissues was studied using radioactive hybridisation on Clontech Cancer Profiling Array II. Hybridization with ubiquitin probe was used for normalisation. The results show that expression of Futrins1-3 is dramatically deregulated in cancerous human tissues (Figure 7). In most of the tumors the expression of Futrins 1-3 is dramatically decreased (colon, stomach, lung, rectum tumors for Futrin1, breast, ovary, bladder, uterus, cervix, rectum tumors for Futrin2, uterus and

cervix tumors for Futrin3). In a few cases the expression of Futrin 1-3 is upregulated (one case of stomach tumor for Futrin1 and 2, ovary tumor for Futrin3). Futrin4 shows very low level of expression in most of the tissues studied, except ovary.

CLAIMS:

1. A diagnostic composition comprising:

(a) at least one nucleic acid molecule comprising the nucleotide sequence encoding Futrin1,2,3 or 4 as depicted in Figure 2; and/or

(b) at least one polypeptide molecule comprising the amino acid sequence encoding Futrin1,2,3 or 4 as depicted in Figure 3; and/or

(c) at least one nucleic acid molecule the complementary strand of which hybridizes to a nucleic acid molecule of (a) and which encodes a polypeptide with the biological activity of Futrin1, 2, 3 or 4; and/or

(d) at least one fragment of (a), (b) or (c) having the biological activity of Futrin1, 2, 3 or 4;

(e) at least one nucleic acid molecule the sequence of which differs from the sequence of the nucleic acid molecule of (a), (c) or (d) due to the degeneracy of the genetic code, and/or

(f) at least one ligand which is capable of specifically binding to the molecule of (a), (b), (c), (d) or (e).

2. The diagnostic composition of claim 1, wherein the ligand is an antibody.

3. The diagnostic composition of claim 1, wherein the nucleic acid molecule of part (d) has a length of at least 10 nucleotides.

4. The diagnostic composition of claim 1 or 2, wherein the ligand is detectably labeled.

5. The diagnostic composition of claim 4, wherein the label is selected from the group consisting of a radioisotope, a

bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.

6. The diagnostic composition of any one of claims 1 to 3, wherein the nucleic acid molecule, polypeptide or ligand are bound to a solid support.

7. Use of a nucleic acid molecule, polypeptide and/or ligand for the preparation of a diagnostic composition as defined in any of claims 1 to 6 for the diagnosis of a disease associated with (a) aberrant expression of *Futrin1, 2, 3 and/or 4* and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 polypeptide.

8. Use according to claim 7, wherein the target to which the nucleic acid molecule hybridizes is a mRNA.

9. A method of diagnosing a disease associated with (a) aberrant expression of *Futrin1, 2, 3 and/or 4* and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 polypeptide in a subject comprising:

(a) determining (a) the amount of expression of *Futrin1, 2, 3 and/or 4* and/or (b) the amount of biologically active Futrin1, 2, 3 and/or 4 polypeptide in a biological sample; and

(b) diagnosing a disease associated with (a) aberrant expression of *Futrin1, 2, 3 and/or 4* and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 polypeptide or a risk for the development of such disease based on an altered amount of expression of *Futrin1, 2, 3 and/or 4* and/or (b) an altered amount of biologically active Futrin1, 2, 3 and/or 4 polypeptide compared to a control.

10. A method for identifying a binding partner to a Futrin1, 2, 3 and/or 4 polypeptide comprising:

(a) contacting said polypeptide with a compound to be screened; and

(b) determining whether the compound effects an activity of said polypeptide or whether binding of the compound to said polypeptide has occured.

11. A method for identifying activators/agonists or inhibitors/antagonists of a Futrin1, 2, 3 and/or 4 polypeptide comprising the steps of:

(a) incubating a candidate compound with said polypeptide;

(b) assaying a biological activity, and

(c) determining if a biological activity of said polypeptide has been altered.

12. A method of identifying and obtaining a drug candidate for therapy of a disease associated with (a) aberrant expression of the gene encoding Futrin1, 2, 3 and/or 4 and/or (b) aberrant activities or amounts of Futrin1, 2, 3 and/or 4 comprising the steps of

(a) contacting a Futrin1, 2, 3 and/or 4 polypeptide or a cell expressing said polypeptide, and optionally the corresponding ligand(s), in the presence of components capable of providing a detectable signal in response to binding to said drug candidate to be screened; and

(b) detecting presence or absence of a signal or increase of the signal generated, wherein the presence or increase of the signal is indicative for a putative drug.

13. An activator/agonist or inhibitor/antagonist of a Futrin1, 2, 3 and/or 4 polypeptide or binding partner of said polypeptide(s) obtainable by the method of any one of claims 10 to 12.

14. A pharmaceutical composition comprising a compound which is capable of modulating the expression of a gene encoding Futrin1, 2, 3 and/or 4 or the activity of Futrin1, 2, 3 and/or

4 and a pharmaceutically acceptable excipient, diluent or carrier.

15. The pharmaceutical composition of claim 14, wherein the compound stimulates expression of the gene encoding Futrin1, 2, 3 and/or 4 or the activity of Futrin1, 2, 3 and/or 4.

16. The pharmaceutical composition of claim 15, wherein the compound is a nucleotide molecule encoding a polypeptide having a biological activity of Futrin1, 2, 3 and/or 4, a Futrin1, 2, 3 and/or 4 polypeptide, an activator/agonist or inhibitor/antagonist of a Futrin1, 2, 3 and/or 4 polypeptide or binding partner of said polypeptide(s) obtainable by the method of any one of claims 10 to 12.

17. Use of a compound as defined in claim 16 for the preparation of a pharmaceutical composition for the treatment of a disease associated with (a) aberrant expression *of* Futrin1, 2, 3 and/or 4 and/or a gene involved into the *wnt* signal cascade and/or (b) aberrant activities or amounts of a Futrin1, 2, 3 and/or 4 and/or polypeptide involved into the Wnt signal cascade.

18. Use according to claim 7 or 17, wherein the disease is a tumor or a disease of the kidneys, muscle, bones and eyes.

19. Use of a nucleotide molecule encoding a polypeptide having a biological activity of Futrin1, 2, 3 and/or 4, a Futrin1, 2, 3 and/or 4 polypeptide, an activator/agonist of a Futrin1, 2, 3 and/or 4 polypeptide or binding partner of said polypeptide(s) for the preparation of a pharmaceutical composition for activating or inhibiting the Wnt signal cascade.

20. Use according to claim 19 for supporting regenerative processes.

Abstract

Compositions for diagnosis and therapy of diseases associated with aberrant expression of *Futrins* and/or wnt

The present invention relates to a composition useful for the diagnosis of diseases associated with aberrant expression of the genes encoding the secreted proteins Futrin1, 2, 3 and/or 4 e.g. in connection with tumors or diseases of the muscle, kidneys or bones. The present invention also relates to a pharmaceutical composition containing a compound which is capable of modifying (a) the expression of the gene encoding Futrin1, 2, 3 and/or 4 or (b) the activity of the Futrin1, 2, 3 and/or 4 protein.

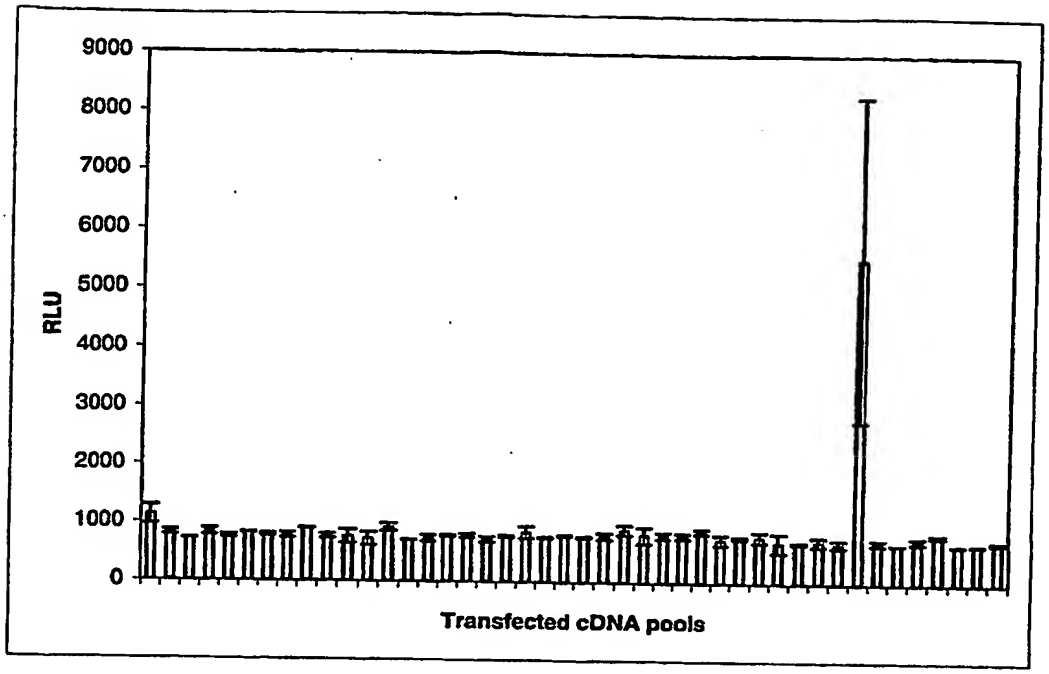


Figure 1

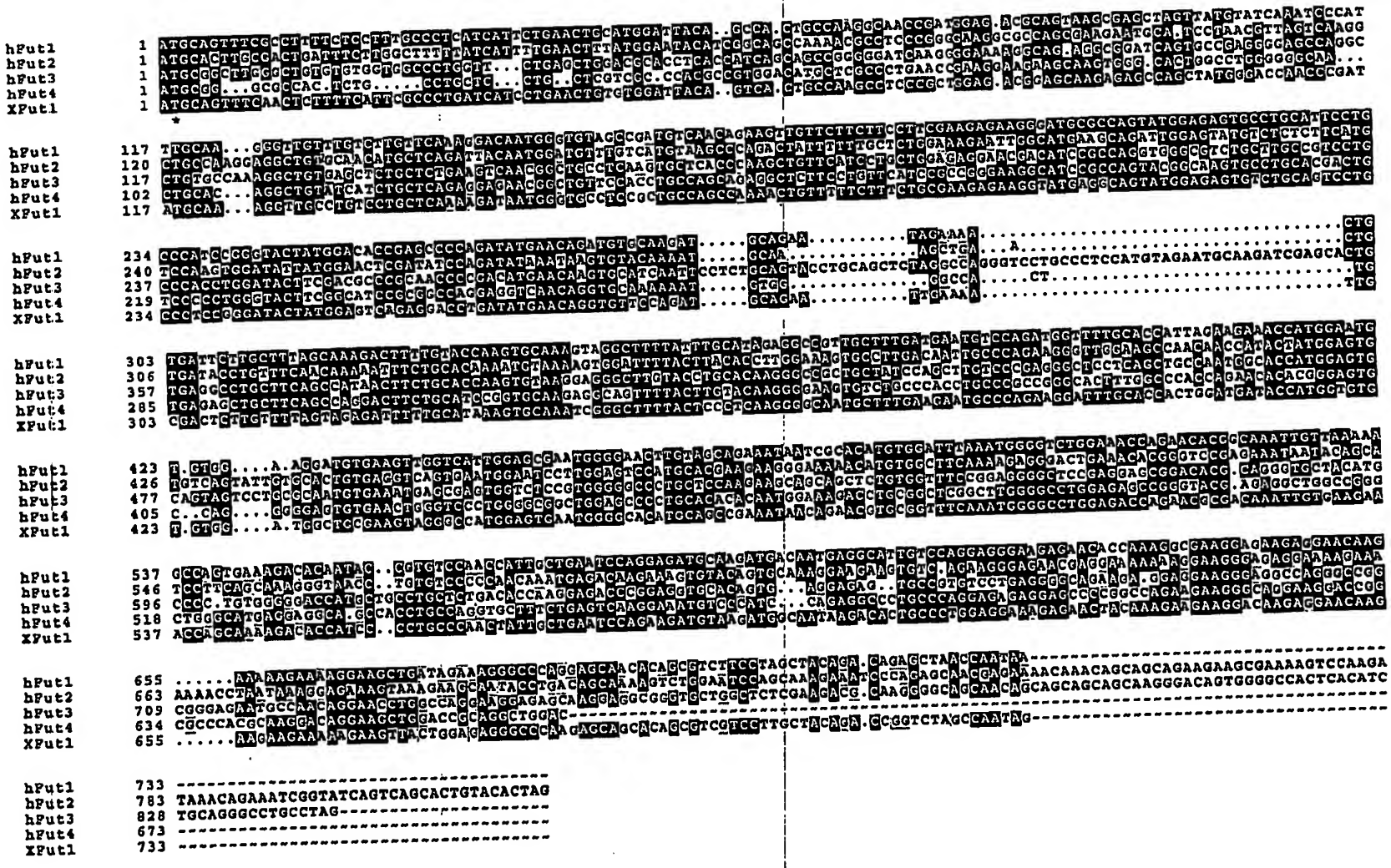
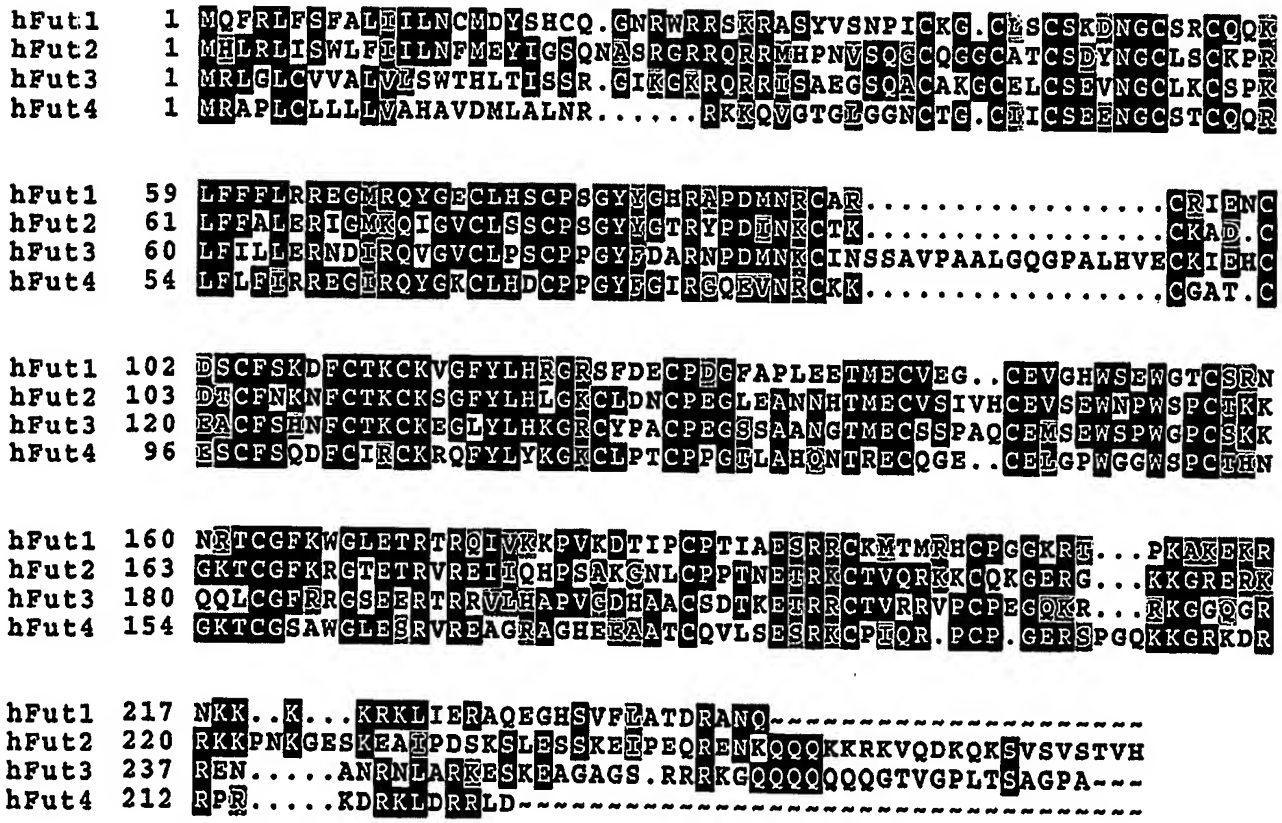


Figure 2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hFut1 59 LFFFLRREGMRQYGECLHSCPSGYYGHRAPDMNRCAR.....CRIENC
hFut2 61 LFFALERIGMKQIGVCLSSCPSGYYGTRYPDLNKCTK.....CKAD.C
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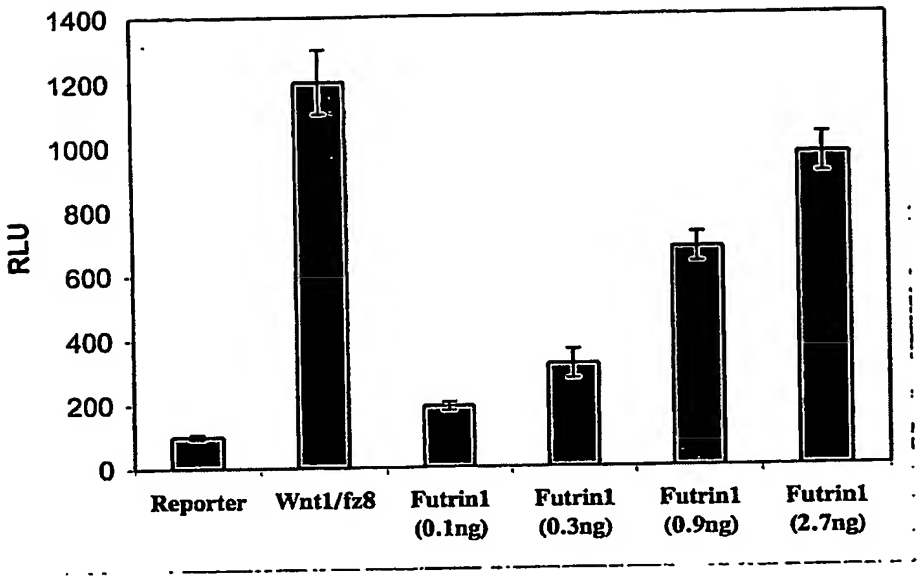
hFut4 54 LFLFLRREGIRQYGKCLHDCPPGYFGIRGQEVNRCKK.....CGAT.C
hFut1 102 DSCFSKDFCTKCKVGFYLHRGRSFDECPDGFAPLEETMECVEG..CEVGHWSEWGTCSRN
hFut2 103 DTCFNKNFCTKCKSGFYLHLGKCLDNCPEGLEANNHTMECVSIVHCEVSEWNPWSPCTKK
hFut3 120 EACFSHNFCTKCKEGLYLHKGRCYPACPEGSSAANGTMECSSPAQCEMSEWSPWGPCSKK
hFut4 96 ESCFSQDFCIRCKRQFYLYKGKCLPTCPPGTLAHQNTRECQGE..CELGPWGGWSPCTHN
hFut1 160 NRTCGFKWGLETRTRQIVKKPVKDTIPCPTIAESRRCKMTMRHCPGGKRT...PKAKEKR
hFut2 163 GKTCGFKRGTETRVREIIQHPSAKGNLCPPTNETRKCTVQRKKCQKGERG...KKGRERK
hFut3 180 QQLCGFRRGSEERTRRVLHAPVGDHAACSDTKETRRCTVRRVPCPEGQKR...RKGGQGR
hFut4 154 GKTCGSAWGLESRVREAGRAGHEAATCQVLSESRKCPIQR.PCP.GERSPGQKKGRKDR
hFut1 217 NKK..K...KRKLIERAQEGHSVFLATDRANQ----------
hFut2 220 RKKPNKGESKEATPDSKSLESSKETPEQRENKQQQKKRKVQDKQKSVSVSTVH
hFut3 237 REN.....ANRNLARKESKEAGAGS.RRRKGQQQQQQQQGTVGPLTSAGPA---
hFut4 212 RPR.....KDRKLDRRLD----------

Figure 3

A


1400
1200
1000
800
600
400
200
0
RLU
Reporter
Wnt1/fz8
Futrin1 (0.1ng)
Futrin1 (0.3ng)
Futrin1 (0.9ng)
Futrin1 (2.7ng)

B

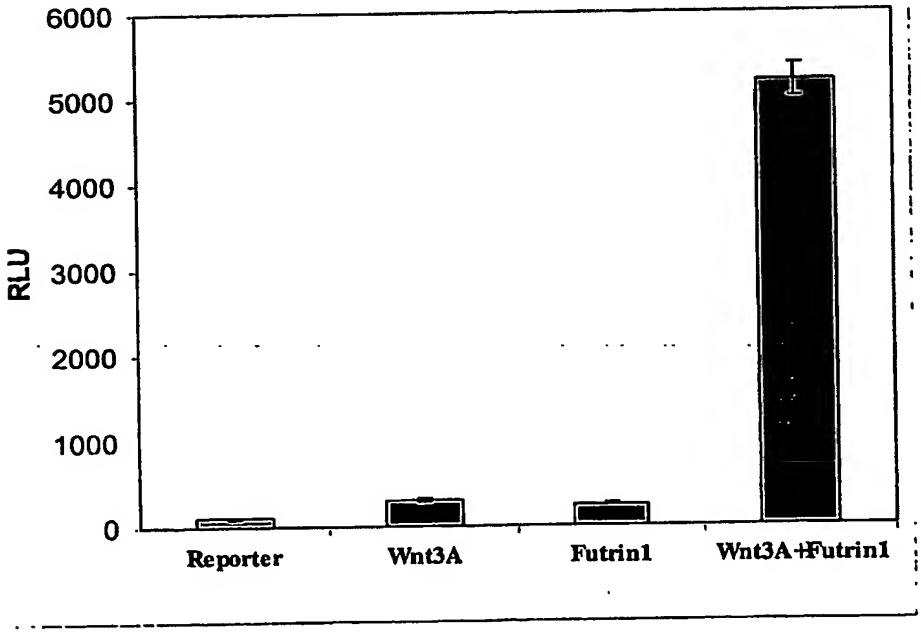
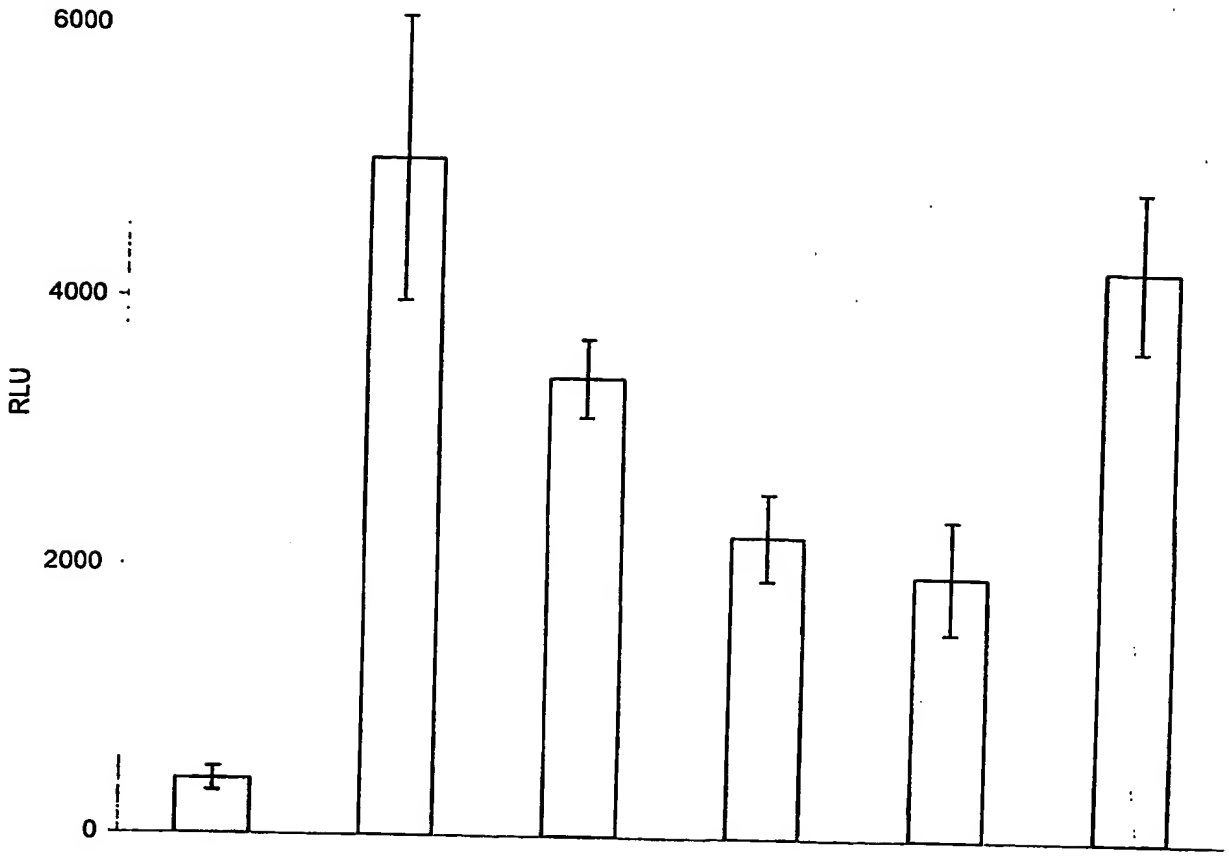

6000
5000
4000
3000
2000
1000
0
RLU
Reporter
Wnt3A
Futrin1
Wnt3A+Futrin1

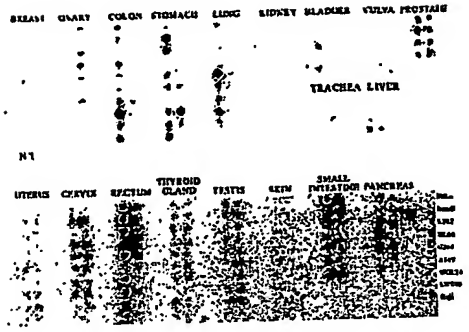
Figure 4



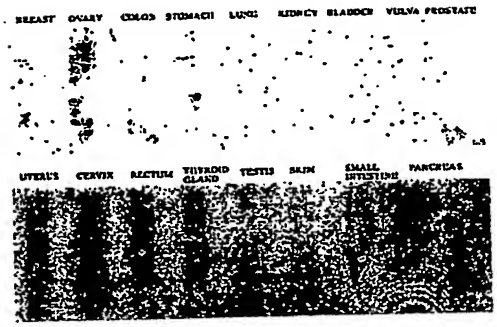
pSuper Non.	+	+				
pSuper hFut1			+		+	+
pSuper hFut2				+	+	+
Wnt3A med.	-	+	+	+	+	+
mFut1						+

Figure 5

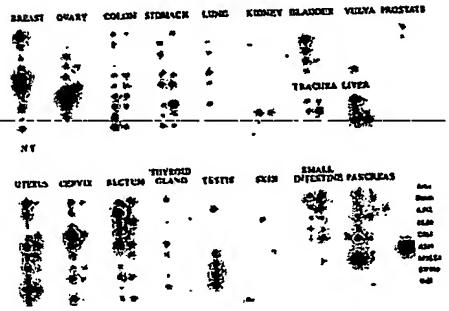
hFutrin1



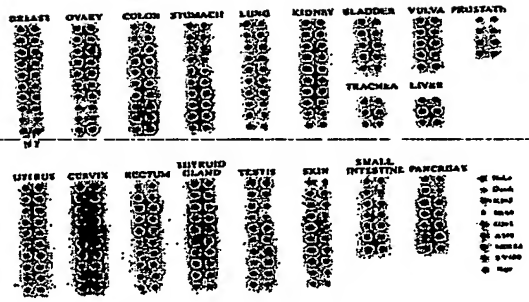
hFutrin3



hFutrin2



Ubiquitin



hFutrin4

Figure 7